

Amendments to the Specification

Please amend the specification as follows.

Page 1, line 10, before the first paragraph, insert the following new paragraph.

-- This application is a divisional application of U.S. patent application serial number 08/882,431, filed June 25, 1997, now Patent Number 6,713,284, issued March 30, 2004. --

Page 10, for the first full paragraph, substitute the following paragraph.

Figure 3. Sequence and secondary structural alignment of bacterial superantigen toxins. Analyses were performed with the applications PILEUP and PROFILE from the Computer Genetics Group (Madison, WI) using sequence data obtained from a variety of sources. Amino acid residue numbering is based on the SEA sequence. (SEQ ID NOS: 17-25).

Page 12, beginning with line 16, after the first full paragraph, insert the following new paragraph.

-- For the invention described below, the following sequence identifiers may be used to refer to certain nucleotide or amino acid sequences. SEQ ID NO:1 refers to a DNA sequence of Staphylococcal enterotoxin A vaccine, periplasmic (A489270P); SEQ ID NO:2 refers to an amino acid sequence of Staphylococcal enterotoxin A vaccine, periplasmic (A489270P); SEQ ID NO:3 refers to a DNA sequence of Staphylococcal enterotoxin A vaccine, cytoplasmic (A489270C); SEQ ID NO:4 refers to an amino acid sequence of Staphylococcal enterotoxin A vaccine, cytoplasmic (A489270C); SEQ ID NO:5 refers to a DNA sequence of Staphylococcal enterotoxin B vaccine (B42360210); SEQ ID NO:6 refers to an amino sequence of Staphylococcal enterotoxin B vaccine (B42360210); SEQ ID NO:7 refers to a DNA sequence of Staphylococcal enterotoxin B vaccine, periplasmic (B899445P); SEQ ID NO:8 refers to an amino sequence of Staphylococcal enterotoxin B vaccine, periplasmic (B899445P); SEQ ID NO:9 refers to a DNA sequence of Staphylococcal enterotoxin B vaccine, cytoplasmic (B899445C); SEQ ID NO:10 refers to an amino acid sequence of Staphylococcal enterotoxin B vaccine,

cytoplasmic (B899445C); SEQ ID NO:11 refers to a DNA sequence for Staphylococcal toxic shock syndrome toxin-1 vaccine (TST30); SEQ ID NO:12 refers to an amino acid sequence for Staphylococcal toxic shock syndrome toxin-1 vaccine (TST30); SEQ ID NO:13 refers to a DNA sequence for Staphylococcal enterotoxin C1 vaccine (SEC45); SEQ ID NO:14 refers to an amino acid sequence for Staphylococcal enterotoxin C1 vaccine (SEC45); SEQ ID NO:15 refers to a DNA sequence for Streptococcal pyrogenic exotoxin A vaccine (SPEA42); and SEQ ID NO:16 refers to an amino acid sequence for Streptococcal pyrogenic exotoxin A vaccine (SPEA42). --

Pages 33, beginning at line 1, please replace the paragraph with the following.

Protein purifications

The appropriate *E. coli* hosts were transformed with plasmids harboring the mutant toxin genes, such as pETA489270C (having accession number ATCC 98449, deposited at the American Tissue Culture Collection (ATCC), Manassas, Virginia under the terms of the Budapest Treaty on June 4, 1997), pETB2360210 (having accession number ATCC 98447, deposited at the ATCC under the terms of the Budapest Treaty on June 4, 1997), pETB899445P (having accession number ATCC 98446, deposited at the ATCC under the terms of the Budapest Treaty on June 4, 1997), and pETB899445C (having accession number ATCC 98445, deposited at the ATCC under the terms of the Budapest Treaty on June 4, 1997). In general, the bacteria were grown to an A600 0.5-0.6 in Terrific Broth (Difco Laboratories, Detroit, MI) containing 50 µg/mL ampicillin or kanamycin. Recombinant proteins were induced with isopropyl-β-D-thiogalactopyranoside (Life Technologies, Gaithersburg, MD) and recovered as cytoplasmic or bacterial periplasmic secretion products. Bacteria were collected by centrifugation, washed with 30 mM NaCl, 10 mM TRIS (pH 7.6), and pelleted by centrifugation and either lysed or osmotically shocked for collection of secreted proteins. Preparations were isolated by CM Sepharose ion-exchange chromatography, rabbit antibody (Toxin Technologies, Sarasota, FL) affinity columns, ion exchange HPLC or similar methods. In some cases partially purified superantigen was further purified by preparative isoelectric focusing (MinipHor; Rainin Instrument Company, Inc., Woburn, MA.). The

MinipHor was loaded with the SEA-enriched fraction from CM Sepharose chromatography in a solution containing 10% (v/v) glycerol and 1% (v/v) pH 6-8 ampholytes (Protein Technologies, Inc., Tucson, AZ). The protein preparations were allowed to focus until equilibrium was reached (approximately 4 hr, 4°C). Twenty focused fractions were collected and aliquots of each were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. The SEA-containing fractions were pooled, and refocused for an additional 4 h. The fractions containing purified SEA were pooled and dialyzed first against 1 M NaCl (48 h, 4°C) to remove ampholytes, and then against PBS (12 h, 4°C). Legitimate amino-terminal residues were confirmed by protein sequencing. Precise measurements of protein concentrations were performed by immunoassay using rabbit antibody affinity-purified with the wild-type superantigens and by the bicinchoninic acid method (Pierce, Rockford, IL) using wild-type protein as standards. All protein preparations were >99% pure, as judged by SDS-PAGE and Western immunoblots. In some cases, as when used for lymphocyte assays, bacterial pyrogens were removed by passing the protein preparations over Polymyxin B affinity columns.